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The Impact of Silver Nanoparticles on Plant Biomass and Chlorophyll Content

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ABSTRACT:*Applications of nanoparticles by industry and its disposal is a new concerned for environment. Depending upon the concentration of particles and its exposure time causes negative impact on V.radiata and B.campestris seedlings. V.radiata was the only species among two test plants which was found to be resistance to Ag nanoparticle and ion solution. Significant inhibition on shoot fresh weight of V.radiata (p=0.008) and B.campestris (p=0.002) was observed at 1000 µg/mL silver nanoparticle solution after treatment period. V.radiata showed significant retardation on dry weight of root at 1000 µg/mL of Ag^+ ions solution after 12^{th} day. The decrease on shoot dry weight with increase in nanoparticle and ion concentration was also observed after 12^{th} day. Exposure to 1000 µg/mL of Ag nanoparticles reported significant retardation on total chlorophyll content V.radiata (p=0.001) and B.campestris (p=0.001) when compare to control after 12^{th} day of treatment. After the treatment period no significant inhibition on chlorophyll ratio was observed when exposed to both Ag nanoparticle and ion solutions. Transmission Electron Microscope reveals breakage of cell wall and vacuoles of test plants which shows the toxic nature of Ag nanoparticles inside treated root cells.*

Key words: *Chlorophyll, Concentration, Nanoparticles, Toxic and Transmission Electron Microscope (TEM).*

I. INTRODUCTION:

Nanotechnology requires control and construction of improved new material at nanoscale level in which integration of nanoscale structures into larger material components and systems take place [1]. Both positive and negative impacts of nanoparticles on higher plants were reported. Nano-SiO_2 and nano-TiO_2 caused an increase in nitrate reductase in *Glycine max* which enhances the ability to absorb and utilize water. However, these two nanoparticles stimulated antioxidant systems and hastened the germination and growth of plants [2]. Effect of silver nanoparticles on reduction of biomass and transpiration rate was also reported in *Cucurbita pepo*. The adverse effect on *C. pepo* was more prevalent in nanoparticles than bulk silver solutions (4.4 to 10 times more) [3]. Magnetic nanoparticles coated with stabilizers such as Tetramethylammonium hydroxide (TMA-OH) was studied on early growth stages of maize plants. Small concentration of aqueous ferrofluid solution added in a culture medium had a stimulating effect on the growth of plants while the enhanced concentration of aqueous ferrofluid solution induced an inhibitory effect. It was found that at low concentration of ferrofluid, there was an increase in chlorophyll "a" level while at higher concentration it was inhibited [4]. Copper nanoparticles of higher concentration (1000 mg/L) caused adverse effect on seedling growth of mung bean. TEM images showed that particles were mostly deposited at 1000 mg/L than 200 mg/L. Copper nanoparticles crossed the cell membrane of *P. radiatus* and *T. aestivum* and aggregated along with the other cellular materials within the cells. The dispersions of nanoparticles resulted in no precipitations in culture plate in this new technique [5]. Bioaccumulation of nanoparticles increased with increase in concentrations of growth media and their bioavailability to test plant was calculated by the bioaccumulation factor. Effect of copper nanoparticles on zucchini plants showed inhibition of root length in seedling compare to control [6].

II. MATERIALS AND METHOD:

2.1 Synthesis of Ag nanoparticles solution:

Silver nanoparticles were synthesized in the aqueous phase, using double distilled water. All reagents were purchased from Merck chemicals and used as received. For Ag nanoparticles preparations, 10^{-3} M $AgNO_3$ solutions were reduced with 10^{-3} M $NaBH_4$ in double distilled water. Tween-20 was added as a surfactant to prevent aggregation of particles. Silver ion solutions were prepared in double distilled water in absence of $NaBH_4$ and Tween-20 [7].

2.2 Seedling growth:

V.radiata and *B.campestris* seeds were selected for the study. The seeds were germinated and uniform seedlings were selected for experiments. The seedlings were grow in Hoagland nutrient solution and transferred in different concentration of nanoparticles and ion solutions at growth chamber. The Phytotoxicity periods continue for 12 days [7].

2.3 Fresh and dry matter estimation:

Fresh weight was measured at different intervals. The treated and untreated seedlings were washed under tap water and then rinsed in distilled water. Roots and shoots were separated and blotted dry. Dry weight was measured by drying root and shoot at 70 ºC for 24 hours in an oven [8].

2.4 Quantifications of Chlorophyll.

Chlorophyll a, b and total chlorophyll was measured by extracting 0.5g of fresh leaf in 3 mL of 80% acetone with a small amount of quartz sand. The homogenate was filtered through Whatman No.1 filter paper. The color intensity was measured at 645 nm and 663 nm using UV-Vis spectrophotometer (Hitachi, Model no.3210).

2.5 Transmission Electron Microscope observations:

Localization of nanoparticles was studied using TEM (Model - JEOL JSM 100 CX). At first segments were taken from the treated seedling roots above the apical part of the root tip. The sample was prepared by standard procedure followed at Sophisticated Analytical Instrument Facility, NEHU, Shillong.

1.6 Statistical analysis:

In every experiment, each treatment was conducted with three replicates. The statistical analysis of experimental values was compared with the control. Statistical significance was done by student–*t* test analysis. It was accepted when the probability of the result by assuming null hypothesis (*p*) is less than 0.05.

III. EXPERIMENTAL FINDING

3.1 Changes in fresh weight of treated and untreated test plants:

The test plants exhibited increase in biomass but at variable rate which depends on plant species, concentration of Ag nanoparticle and ions and its exposure time. No significant reduction on root fresh weight was reported after 1st day of treatment on *V.radiata* and *B.campestris* by Ag nanoparticle and ion solution. No significant inhibition on root fresh weight was observed at 50µg/mL and 500 µg/mL of Ag nanoparticle solution in test plants. Concentration of Ag ions (50 µg/mL, 500 µg/mL and 1000 µg/mL) exposed to test plants did not show significant inhibition on root fresh weight till 3rd day. Similar result was obtained when ZnO nanoparticle (1 ppm and 20 ppm) resulted in an increase in root and shoot biomass of mung and gram seedling. The increase in biomass at 1 ppm and 20 ppm concentration suggests the optimum dose limit for the growth of mung and gram seedlings [9].

Adverse effects on root fresh weight was observed from 6th day onwards by both nanoparticle and ion treatment. Effect on root fresh weight was observed beyond 50 µg/mL of Ag nanoparticle concentration in test plants. Significant reduction on root fresh weight was observed at 500 µg/mL in *V.radiata* (p=0.027) and *B.campestris* (p=0.024) compared to control after 6th day. However 1000 µg/mL of Ag nanoparticle solution reported adverse effect on root fresh weight of, *V.radiata* (p=0.005) and *B.campestris* (p=0.013) compared to control. Ag^+ ion solution showed significant inhibition at 500 µg/mL on fresh weight of root in *B.campestris* (p=0.027). 1000 µg/mL of Ag^+ ion showed significant retardation on fresh weight of root in *V.radiata* (p=0.012) and *B.campestris* (p=0.006) compared to control. There was a significant reduction on root fresh weight at 500 µg/mL and 1000 µg/mL of Ag nanoparticle solution in *B.campestris* plants. At 1000 µg/mL Ag^+ ion solution, significant reduction on root fresh weight was observed in *V.radiata* (p=0.017) compared to control root. No significant effect was observed in *B.campestris* at 50 µg/mL, 500 µg/mL and 1000 µg/mL concentration of silver ions after 9th day. *V.radiata* was the only species among two test plants which was found to be resistance to Ag nanoparticle and ion solution after 12th day (Fig.1). However, *B.campestris* reported adverse effect on root fresh weight by Ag nanoparticle solution (at 500 µg/mL and 1000 µg/mL) and Ag^+ ion solution (at 1000 µg/mL) after 12th day.

The Fresh weight of shoot in test plants remained unaffected by the experimental values. No significant effect was observed on shoot fresh weight of test plants by both Ag nanoparticle and ion solution. However, the influence on shoot fresh weight was clearly observed from 3rd day by both Ag nanoparticle and ion solutions. Similar to the patterns of root fresh weight, the shoot fresh weight was also not affected at low concentration i.e. 50 µg/mL of Ag nanoparticle and ion solution after 3rd day. *B.campestris* showed significant retardation on shoot fresh weight at both 500 µg/mL (p=0.048) and 1000 µg/mL (p=0.017) of Ag nanoparticle solution compared to control. While *V.radiata* reported significant inhibition on shoot fresh weight at 1000 µg/mL (p=0.015) of Ag nanoparticle solution after 3rd day. Among Ag^+ ion solution, 1000 µg/mL showed significant inhibition on shoot fresh weight in *V.radiata* (p=0.043) when compared with control.

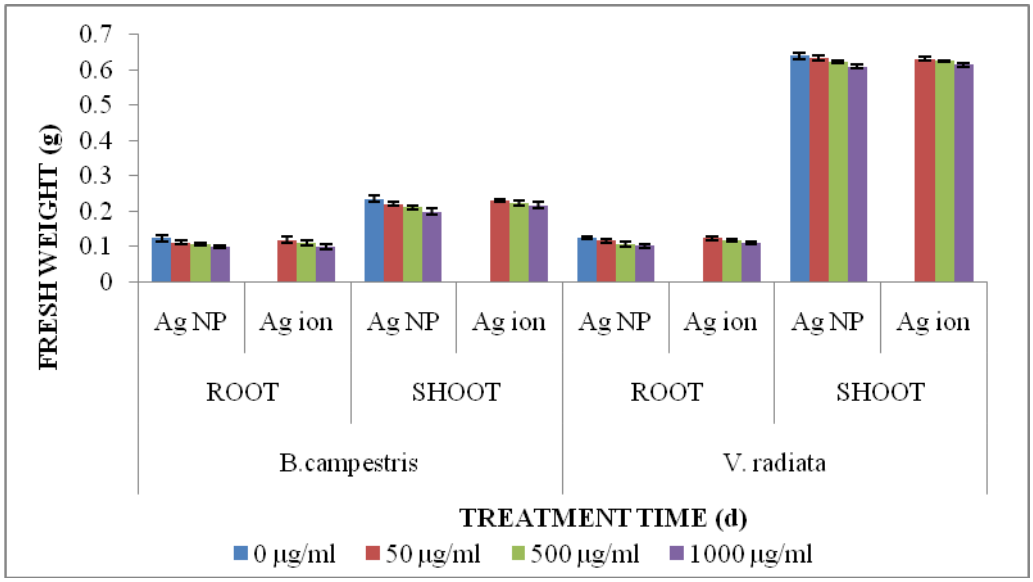


Fig 1: Effect of Ag nanoparticles and *V.radiata* and *B.campestris* biomass (fresh weight) after 12 days of treatment.

Adverse effect on shoot fresh weight was observed from 6th day onwards. It was found that shoot fresh weight of *V.radiata* shows resistance to both Ag nanoparticle and ion treatments. *B.campestris* showed significant inhibition on shoot fresh weight at 500 µg/mL (p=0.001) and 1000 µg/mL (p=0.000) of Ag nanoparticle solutions when compared with control. Significant retardation on shoot fresh weight was also observed in *B.campestris* (p=0.012) by 1000 µg/mL of Ag^+ ion solution compare to control after 6th day. *B.campestris* shoot fresh weight was significantly inhibited after 9th day at 500 µg/mL and 1000 µg/mL of Ag nanoparticle solution. Significant retardation was observed in *V.radiata* (p=0.026) and *B.campestris* (p=0.030) at 1000 µg/mL of Ag^+ ion solution after 9th day. Significant inhibition on shoot fresh weight of *V.radiata* (p=0.008) and *B.campestris* (p=0.002) was observed at 1000 µg/mL Ag nanoparticle solution compared to control after 12th day of treatment (Fig.1). Similar result was obtained when ZnO nanoparticle of 2000 ppm caused decrease in biomass of mung and Gram seedling. Decrease in biomass of root and shoot shows the toxic nature of ZnO nanoparticle beyond 20 ppm concentration [9]. It was reported that decreased in fresh weight by silver ion was probably due to increase in metabolic activities in sunflower seedling [10]. Adverse effect of silver ions on fresh weight of sunflower plant supports our results since there was a decline in fresh weight of root and shoot by both Ag nanoparticle and ion solution during treatment period.

3.2 Changes in dry weight of treated and untreated test plants:

The influence of Ag nanoparticle and ion on dry weight of root of test plants after treatment period was shown in Fig.2. Dry weight production of root of test plants was not inhibited by any concentration of the Ag nanoparticle and ion treatment on 1st day. On the contrary, the test plants exhibited increase in dry weight with time, but at different time rate. *V.radiata* showed significant retardation on dry weight of root at 1000 µg/mL of Ag nanoparticle solution after 3rd day of treatment. No significant inhibition on root dry weight was observed in *B.campestris* at 500 µg/mL (p=0.110) and 1000 µg/mL (p=0.102) of Ag nanoparticle solutions when compared to control. There was no adverse effect on root dry weight in *V.radiata* and *B.campestris* seedling by Ag^+ ion solution after 3rd day. Significant inhibition on dry weight of root was reported in *B.campestris* at 500 µg/mL and 1000 µg/mL of Ag nanoparticle solution. The dry weights of roots of test plants were adversely affected by all concentration (50 µg/mL, 500 µg/mL and 1000 µg/mL) of Ag nanoparticle solution after 9th day. Dry weight of *B.campestris* root showed significant inhibition at 50 µg/mL (p=0.029), 500 µg/mL (p=0.004) and 1000 µg/mL (p=0.000) of nanoparticle concentrations compare to control. Similarly *V.radiata* resulted in significant retardation of root dry weight at 50 µg/mL (p=0.002), 500 µg/mL (p=0.001) and 1000 µg/mL (p=0.000) of nanoparticle solutions when compare with control after 9th day of treatment. *V.radiata* showed significant retardation on dry weight of root at 50 µg/mL (p=0.050), 500 µg/mL (p=0.008) and 1000 µg/mL (p=0.003) of Ag nanoparticles solution when compared to control after 12th day. *B.campestris* also reported significant inhibition at 50 µg/mL (p=0.037), 500 µg/mL (p=0.002) and 1000 µg/mL (p=0.000) on dry weight of root compared to control. *V.radiata* showed significant retardation on dry weight of root at 1000 µg/mL of Ag^+ ions solution after 12th day.

Fig.2 shows the dry weight of shoot of test plants as affected by the application of various concentration of Ag nanoparticle and ion solution after the treatment period. No significant effect on dry weight

of shoot was observed in all test plants after 1st day of treatment. Ag nanoparticle concentration beyond 50 µg/mL resulted in significant inhibition on shoot dry weight in *V.radiata* and *B.campestris* after 3rd day. 500 µg/mL of Ag nanoparticle solution showed significant retardation in *V.radiata* (p=0.026) and *B.campestris* (p=0.007) shoot dry weight compare to control. 1000 µg/mL of Ag nanoparticle solution resulted in significant retardation on shoots dry weight in *V.radiata* (p=0.005) and *B.campestris* (p=0.002) when compare with control after 3rd day. Biomass reduction was more observed after 6th day in *B.campestris* compared to *V.radiata* when exposed to Ag nanoparticle solution. 50 µg/mL (p=0.004), 500 µg/mL (p=0.001) and 1000 µg/mL (p=0.000) of Ag nanoparticle solution showed significant inhibition on dry weight of *B.campestris* shoot after 6th day of treatment. The test plants exhibited increase or decrease in shoot dry weight with exposure time and concentration. Significant retardation on shoot dry weight was observed at 1000 µg/mL of Ag nanoparticle in *B.campestris* seedling after 9th day.

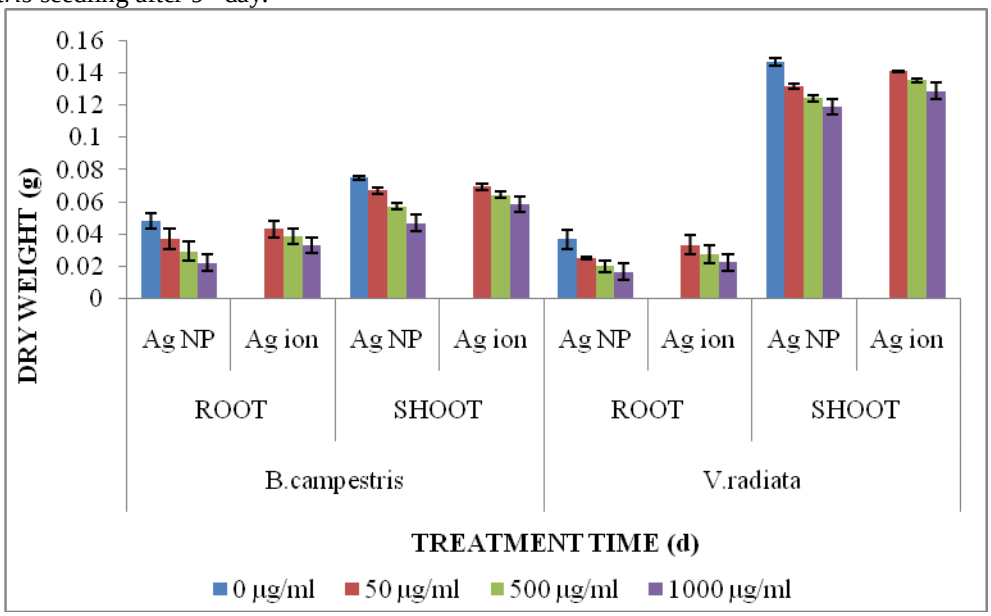


Fig 2: Effect of Ag nanoparticles and ions on *V.radiata* and *B.campestris* biomass (dry weight) after 12 days of treatment.

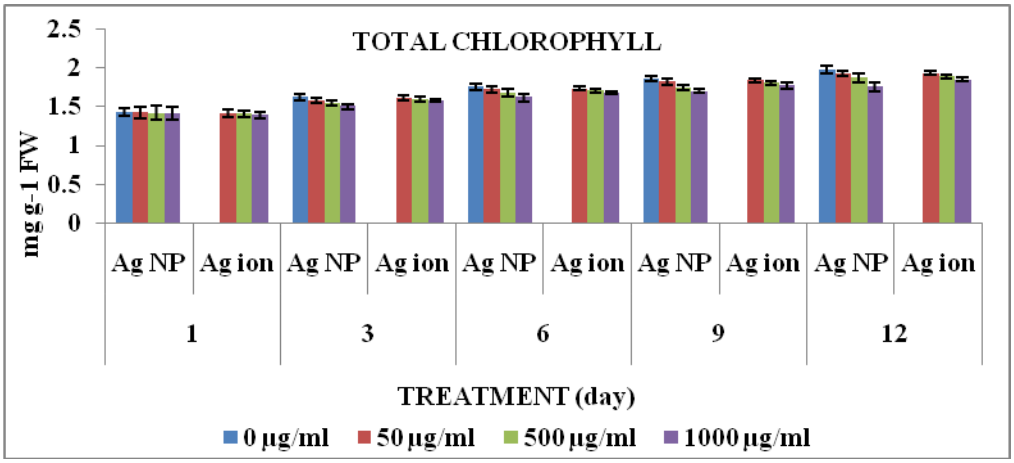
The decrease on shoot dry weight with increase in Ag nanoparticle and ion concentration was also observed after 12th day. 500µg/mL (p=0.012) and 1000 µg/mL (p=0.004) of nanoparticle solution showed significant retardation on dry weight of *V.radiata* shoot compared to control after 12th day. Significant changes on dry weight of *Lemna minor* L at different Ag nanoparticle concentrations are due to different nanoparticle size [11]. Similar result was also observed when 1000 µg/mL nanoparticle concentration showed more adverse effect on dry weight of test plants than 50 µg/mL and 500 µg/mL Ag nanoparticle solution.

3.3 Estimation on chlorophyll content of seedlings:

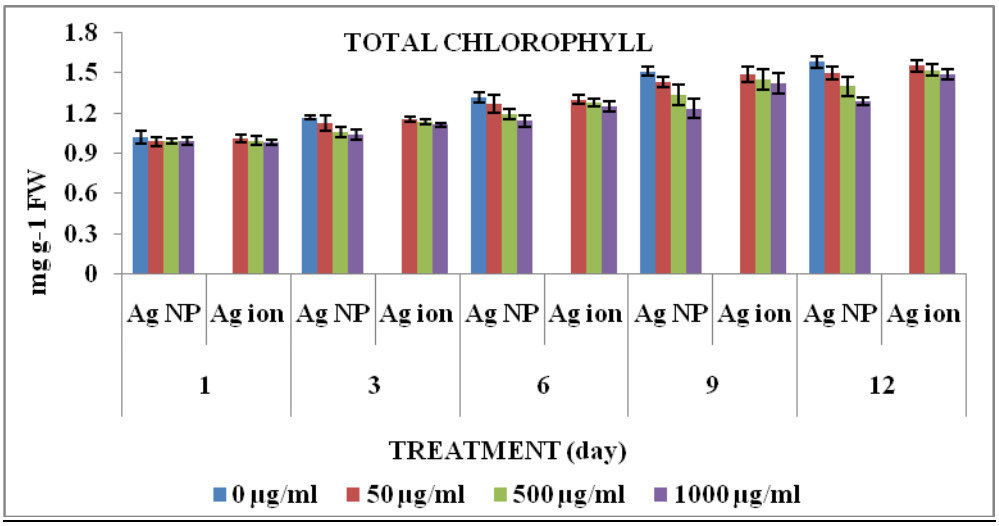
Effect on total chlorophyll content exposed to different concentration of Ag nanoparticle and ion solutions was observed in *V.radiata* and *B.campestris* plants (Fig. 3A and Fig. 3B). The chlorophyll content of different crop plants was tolerant to both Ag nanoparticle and ion concentration used and, therefore, chlorophyll production was not affected till 3rd day. . It was reported that Ag nanoparticles of 20 nm taken up by plants which were mostly in intracellular spaces could be transported inside plant cells through plasmadesmata of root cells [12]. These nanoparticles were then pass through shoots and then accumulated on leaves which caused adverse effect on total chlorophyll content of test plants. The total chlorophyll contents at 50 µg/mL of both Ag nanoparticle and ion solution did not showed any significant inhibition in test plants. A study reported that chlorophyll content of maize plants was found to be increased by low concentration (10-50 µl/L) while it was found to be inhibited by higher concentrations of magnetic nanoparticle [13]. *B.campestris* showed significant inhibition on total chlorophyll content at 500 µg/mL (p=0.044) and 1000 µg/mL (p=0.018) of Ag nanoparticle solutions compared to control. However *V.radiata* showed significant inhibition on total chlorophyll content at 1000 µg/mL (p=0.017) of Ag nanoparticle solutions compared to control. 1000 µg/mL (p=0.024) of Ag^+ ion solutions` showed significant retardation on total chlorophyll content of *B.campestris*. Adverse effect was observed at 1000 µg/mL of Ag nanoparticles in *V.radiata* (p=0.033) and *B.campestris* (p=0.010) on total chlorophyll content compare to control. Higher concentration of Ag^+ ion i.e. 1000 µg/mL resulted in significant inhibition in total chlorophyll content of *V.radiata* (p=0.002) and *B.campestris* (p=0.001) when compared to

control after 9th day. Moreover after 9th day of treatment Ag^+ ion solution showed significant inhibition in total chlorophyll content of *V.radiata* (p=0.038) compare to control. Increase in concentration of Ag nanoparticle showed significant effect on *V.radiata* and *B. campestris* after 12th day. It was observed that 500 µg/mL of nanoparticle solution shows significant retardation on total chlorophyll content in *V.radiata* (p=0.050) and *B.campestris* (p=0.010) compare to control. Exposure to 1000 µg/mL of Ag nanoparticles reported significant retardation on total chlorophyll content *V.radiata* (p=0.001) and *B.campestris* (p=0.001) when compare to control after 12th day of treatment. *V.radiata* showed significant inhibition on total chlorophyll content at 500 µg/mL (p=0.012) and 1000 µg/mL (p=0.002) Ag^+ ion solution when compared to control

Fig. 4 shows the effect on chlorophyll ratio of test plants by both Ag nanoparticle and ion solutions. Decrease in chlorophyll ratio was observed in *B.campestris* with increase in concentration of nanoparticle solution. 500 µg/mL of Ag^+ ion solution resulted in high chlorophyll ratio in *V.radiata* plants compared to 50 µg/mL and 1000 µg/mL Ag^+ ion concentrations. Increased in chlorophyll ratio was observed with increase in nanoparticle concentration in *V.radiata* after 3rd day. However *B.campestris* showed a decrease in chlorophyll ratio after 3rd day at 1000 µg/mL (1.859±0.078) compared to 500 µg/mL (1.918±0.102) Ag nanoparticle solutions. In *V.radiata*, 500 µg/mL of Ag^+ ion solution showed a decrease in chlorophyll ratio compared to 50 µg/mL and 1000 µg/mL of Ag^+ ion concentrations. It was observed that chlorophyll ratio decrease with increase in concentration of Ag^+ ion solutions. However *B.campestris* showed an increase in chlorophyll ratio with increase in Ag^+ ion concentrations after 3rd day. Chlorophyll ratio was found to be decreased with increase in Ag^+ ion concentration in *V.radiata* seedlings after 6th day.



A



B

Fig 3: Effect of Ag nanoparticles and ions on total chlorophyll content of (A) *V.radiata* and (B) *B.campestris* in Hoagland nutrient solution during 12 days of treatment.

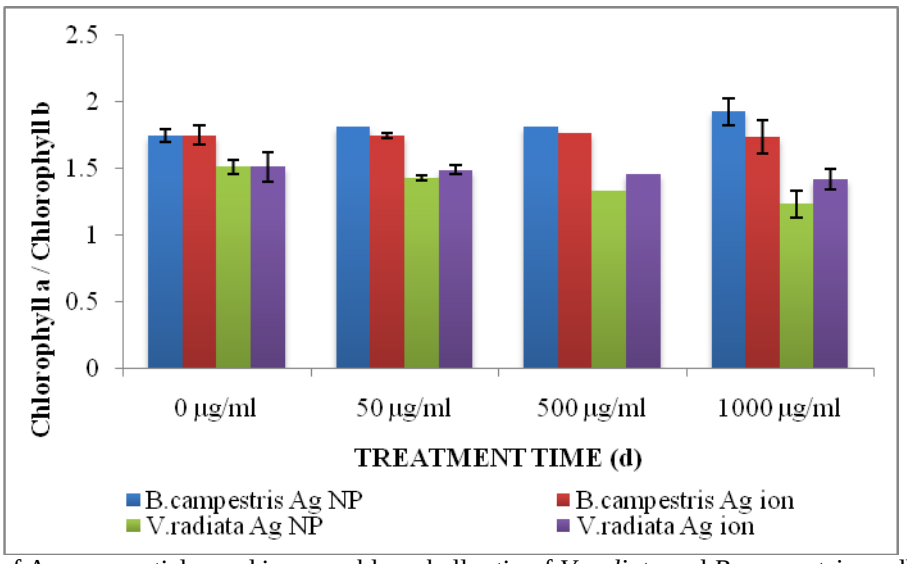
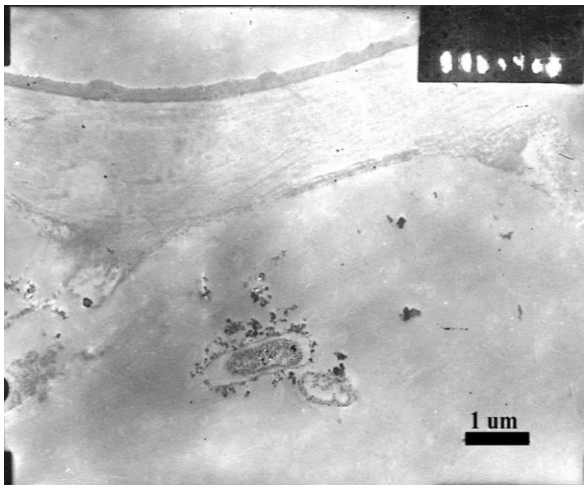


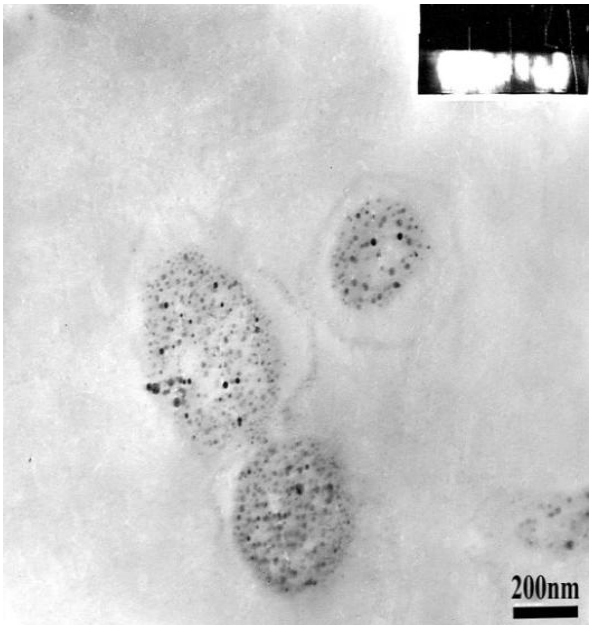
Fig 4: Effect of Ag nanoparticles and ions on chlorophyll ratio of *V.radiata* and *B.campestris* seedlings after 12 days of treatment.

B.campestris showed a decrease in chlorophyll ratio after 9th day at 1000 µg/mL compared to 50 µg/mL and 500 µg/mL of Ag^{+} ion solutions. However after 12th day of the treatment period no significant inhibition on chlorophyll ratio was observed on exposure to both Ag nanoparticle and ion solutions. Increase in LHC II content help to promotes energy transfer and oxygen evolutions in photosystem II in spinach [14]. It was also reported that increase in Hill reactions and activity on chloroplasts by nano-TiO_2 resulted in an acceleration of FeCy reduction and oxygen evolution in *Spinacia oleracea*. [15, 16]. Thus we can assume that Ag nanoparticle and ion solution at higher concentration (500 µg/mL and 1000 µg/mL) may directly affect the LHC II content on thylakoid membrane of selected test plants. This will increase the Hill reactions and activity on chloroplasts of *V.radiata* and *B.campestris* leaves.

3.4 TEM observations:

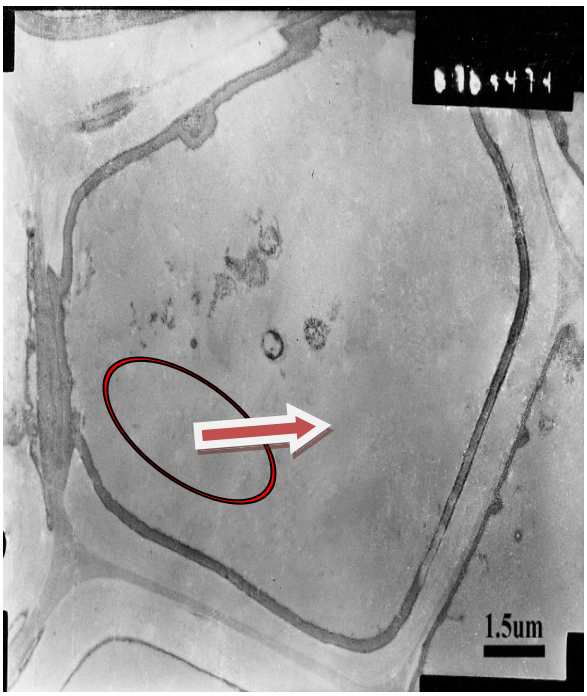
Fig. 5 and Fig. 6 show detection of silver nanoparticles inside the root tissue of both *V.radiata* and *B.campestris*. The observation from the micrographic image (Fig.6) indicated that the whole cell and its intracellular portion i.e. plasmadesmata have silver nanoparticle particle. Magnified image of whole cell showed presence of individual and aggregated Ag particles which were clearly visible inside the cytoplasm of cell.

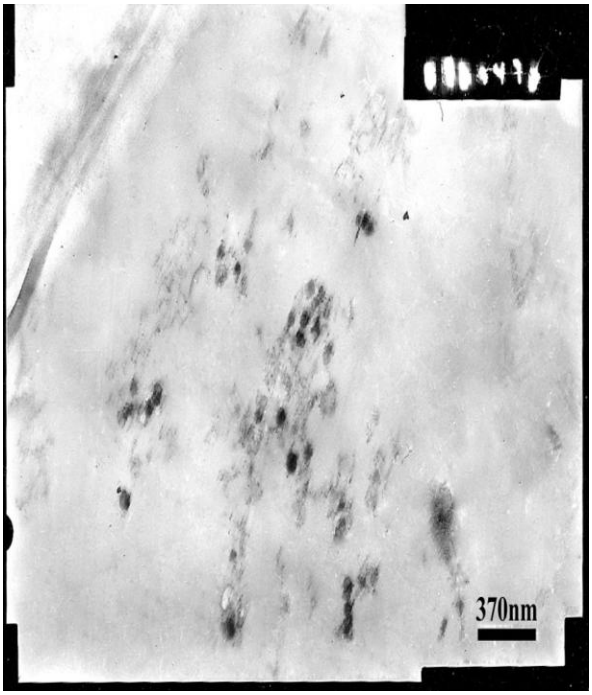




A **B**

Fig 5: TEM images of the roots of *V.radiata* exposed to Ag nanoparticle of 1000 µg/mL showing (A) Depositions of nanoparticles inside the cell, (B) Ag nanoparticles inside vacuoles.





A **B**

Fig 6: TEM images of the roots of *B.campestris* exposed to Ag nanoparticle of 1000 µg/mL showing (A)Deposition of nanoparticle inside whole cell , (B) Magnified portion of image A showing accumulation of particles in plasmadesmata and cell wall.

Accumulation of Ag nanoparticle was clearly observed inside vacuoles of root cell (Fig.5). Deposition of both individual and aggregate particle was found inside the cell wall which indicates the penetration of Ag particle inside the cells. The diameter of Ag nanoparticles was measured inside the plant cell and was found to be around 20 nm in size. The nanomaterials were found to be spherical in shape. One important hypothesis was established regarding transportation of smaller particles inside the cells. Cell walls thickness of about 5 to 20 nm functions act as natural sieves which transports small nanoparticles passes through large pores to enter in the protoplasm. New and large pores were created for passaging of larger nanoparticles at the cell wall [17, 18].

IV. CONCLUSION:

Research on nanoparticles has received a great deal of interest in every discipline. Its applications can be found in many areas due to its high demand. But its adverse effect is always a concern for our environment. It penetrates easily inside the plant cells and causes effects on biomass and chlorophyll content. Deposition of small size nanoparticles inside the cell wall and vacuoles causes disturbance in metabolic activity of plants. The effect can be minimizing by limiting the concentration of nanoparticles solution used in different activity. *V.radiata* and *B.campestris* were both economically important plants, nanoparticles can easily find their way in human body through food chain. More investigations are needed to determine the negative impact of nanoparticles on crop plants and its consequences in other living organisms.

V. ACKNOWLEDGMENT:

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